Glyceraldehyde 3-Phosphate:NADP⁺ Reductase of Spinach Leaves

Steady State Kinetics and Effect of Inhibitors

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ABSTRACT

The steady state kinetics of glyceraldehyde 3-phosphate:NADP⁺ oxidoreductase (GNR) (EC 1.2.1.9) have been investigated. The enzyme exhibits hyperbolic behavior over a wide range of substrate concentrations. Double-reciprocal plots are nearly parallel or distantly convergent with limiting $K_m$ values of 2 to 5 micromolar for NADP⁺ and 20 to 40 micromolar for D-glyceraldehyde 3-phosphate (G3P). The velocity response to NADP⁺ as the varied substrate is however sigmoidal if G3P concentration exceeds 10 micromolar, whereas the response to G3P may show inhibition above this concentration. This ‘G3P-inhibited state’ is alleviated by saturating amounts of NADP⁺ or NADPH. Product inhibition patterns indicate NADPH as a potent competitive inhibitor to NADP⁺ (K, 30 micromolar) and mixed inhibitor towards G3P, and 3-phosphoglycerate (3PGA) as mixed inhibitor to both NADP⁺ and G3P (K, 10 millimolar). The data, and those obtained with dead-end inhibitors, are consistent with a nonrapid equilibrium random mechanism with two alternative kinetic pathways. Of these, a rapid kinetic sequence (probably ordered with NADP⁺ binding first and G3P binding as second substrate) is dominant in the range of hyperbolic responses. A reverse reaction with 3PGA and NADPH as substrates is unlikely, and was not detected. Of a number of compounds tested, erythrose 4-phosphate (K, 7 micromolar) and Pi (K, 2.4 millimolar) act as competitive inhibitors to G3P (uncompetitive towards NADP⁺) and are likely to affect the in vivo activity. Ribose 5-phosphate, phosphoenolpyruvate, ATP, and ADP are also somewhat inhibitory. Full GNR activity in the leaf seems to be allowed only under high photosynthesis conditions, when levels of several inhibitors are low and substrate is high. We suggest that a main function of leaf GNR is to supply NADPH required for photospiration, the reaction product 3PGA being cycled back to chloroplasts.

Most of the carbon assimilated during plant photosynthesis is exported from chloroplasts as triose phosphate by the phosphate carrier (15) to be converted to sucrose in the cytoplasm. Some triose phosphate, however, may be oxidized to 3-phosphoglycerate by two cytosolic enzyme systems acting in parallel (19, 23): triose phosphate dehydrogenase (NAD-

G₃P⁻ + NADP⁺ → 3PGA²⁻ + NADPH + 2H⁺

Scheme 1

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1 Abbreviations: 3PGA, 3-phosphoglycerate; E4P, erythrose 4-phosphate; G3P, D-glyceraldehyde 3-phosphate; GNR, glyceraldehyde 3-phosphate:NADP⁺ reductase; NER, nonrapid equilibrium random.

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have also been examined. It is suggested that leaf GNR is often inhibited in vivo by cellular metabolites, and de-inhibited under photosynthetic conditions.

MATERIALS AND METHODS

Enzyme Purification

GNR was purified essentially as previously described (22) with some improvements. Spinach (Spinacia oleracea L.) leaves (usually 200 g) were homogenized on ice in a Servall Omnimixer in 2 volumes of prechilled buffer A (40 mM Tris-HCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, pH 8). Subsequent operations were carried out in the cold room or on ice if not stated otherwise. After centrifugation (20 min, 20,000 g), crystalline ammonium sulfate was dissolved in the supernatant fluid to 50% saturation, and the precipitate was discarded by centrifugation. This second supernatant was brought to 60% saturation with (NH₄)₂SO₄ and centrifuged. The precipitate was dissolved in buffer A to 20 ml, and an equal volume of acetone at −18°C was added in drops. The precipitate of the subsequent centrifugation (−18°C, 20,000 g, 30 min) was resuspended in buffer A and clarified (20 min, 20,000 g). The supernatant was desalted through Sephadex G-25 (2.6 × 30 cm) equilibrated with buffer B (10 mM Tris-HCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, pH 7.5). The protein at the excluded volume was loaded on a DEAE cellulose column (DE 52, 1 × 20 cm) equilibrated with buffer B, and a linear KCl gradient (0-0.2 M) was developed overnight. GNR eluted as a single peak around 80 mM KCl. The more active fractions were treated with NADP⁺ (0.05 mM), concentrated (Amicon 52 cell, PM 10 membrane) and applied to a Sephadex G-200 column (2.6 × 60 cm) equilibrated with buffer C (10 mM Tris-HCl, 1 mM EDTA, 3 mM 2-mercaptopethanol, 100 mM KCl, 0.1 mM NAD⁺, pH 8), GNR eluted as a single peak. The preparation containing the more active fractions from this step is purified GNR. It is concentrated and dialyzed against buffer B, and conserved at −20°C up to 1 year in the presence of NADP⁺, glycerol, and Pi as described in "Results." This preparation contains a major 53-kD polypeptide (22). Fourteen preparations have been obtained by this method.

Chemicals

Chemicals were obtained mainly from Sigma. D-G3P was the only substrate used, since the L-form is inhibitory (19).

| Table 1. Purification Scheme of GNR From 200 g Spinach Leaves |
|------------------|-----|------------------|
|                  | Activity | Protein |
|                  | μmol min⁻¹ | mg    | μmol min⁻¹ | Purification |
| Homogenate       | 63.9        | 2648 | 0.024       | 1 |
| (NH₄)₂SO₄        | 39.6        | 117  | 0.338       | 14 |
| Acetone          | 31.3        | 25   | 1.25        | 52 |
| DEAE cellulose   | 11.1        | 0.85 | 13.1        | 550 |
| Sephadex G-200   | 9.4         | 0.40 | 23.5        | 980 |

The G3P was obtained according to instructions by the producer from the diethyl acetal (dicyclohexylammonium salt) by passage through Dowex AG 50W-4, previously washed several times with 1 N HCl, then water. G3P concentration was determined in the presence of 1 mM NAD⁺ using NAD-triose phosphate dehydrogenase from rabbit muscle.

Assay

GNR was assayed, if not otherwise stated, in 100 mM Tricine-KOH, 1 mM EDTA, 0.10 mM NAD⁺, 0.30 mM G3P (added last), pH 8.0, 25°C. Assays were monitored at 340 nm in a thermostated Jasco Uvidec spectrophotometer equipped with computer facility. We found that G3P was stable for several min in reaction mixture, at variance from some reports (10). Velocity was measured during the linear, steady-state phase of the reaction following an initial lag (1-3 min). Under these conditions velocity was proportional to protein concentration. Kinetic assays were performed with 0.05 to 0.2 μg of protein. Activity is expressed as nmol NAD⁺ reduced min⁻¹ ml⁻¹ of diluted enzyme (5 μg ml⁻¹). Most results shown are from a single enzyme preparation, but each of them was
Table II. Storage Conditions and Stability of GNR

<table>
<thead>
<tr>
<th>Additions</th>
<th>Relative Activity % of initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>NADP+, 0.1 mM</td>
<td>83</td>
</tr>
<tr>
<td>Pi, 5 mM</td>
<td>9</td>
</tr>
<tr>
<td>Glycerol, 10% (v/v)</td>
<td>92</td>
</tr>
<tr>
<td>NADP+ + Pi</td>
<td>97</td>
</tr>
<tr>
<td>NADP+ + glycerol</td>
<td>99</td>
</tr>
<tr>
<td>Pi + glycerol</td>
<td>94</td>
</tr>
<tr>
<td>NADP+ + Pi + glycerol</td>
<td>100</td>
</tr>
</tbody>
</table>

obtained with at least another preparation. The pH/activity profile was studied in Mops-Tricine buffer, 40 mM each, adjusted to pH with KOH. The pH did not change with addition of substrates or during reaction.

RESULTS

The GNR was purified from spinach leaves by a method (Table I) derived from Pupillo and Faggiani (22). This preparation is electrophoretically homogeneous (Fig. 1), with higher specific activity than reported previously (16, 22), up to 25 μmol min⁻¹ mg⁻¹ protein. The procedure is reproducible and can yield up to 0.2 mg pure protein per 100 g of leaves. The enzyme is quite sensitive to freezing and thawing, but it is protected by NADP⁺ or glycerol (Table II). It is completely stable for at least 1 year if stored at -20°C in buffer B (see "Materials and Methods") containing 10% glycerol (v/v), 0.10 mM NADP⁺, and 5 mM K-phosphate. When again equilibrated with buffer C, GNR can be kept for 1 to 2 weeks at 2 to 4°C with little loss of activity.

GNR activity was studied in the pH range 6 to 9. Maximum activity was found around pH 8.2 when substrates were saturating, but the center of the peak was shifted towards lower values when substrates were nonsaturating (Fig. 2). The pH/activity curve was steep between pH 7 and 8 and declined at higher pH values. Little activity was observed below pH 7, where the reaction was relatively insensitive to G3P concentration. In fact, both Kᵣ (G3P) and Vₘₐₓ increased with increasing pH (not shown). Values of Kᵣ NADP⁺ did not change markedly with pH (all below 10 μM).

GNR kinetics were investigated in numerous preparations at different pH values, although the experiments reported here are based on a single preparation at pH 8.0. Substrate/velocity curves were hyperbolic, and double-reciprocal plots derived from them were linear when NADP⁺ was varied at G3P concentrations below 5 μM (Figs. 3A and 4). However, the plots were partly sigmoidal when the nonvaried substrate G3P was increased above 10 μM (Fig. 3A), i.e. upwards concave in double-reciprocal plots (Fig. 4). Hill coefficients were up to 2.3. The linear segment of the NADP⁺ pattern became progressively shorter with increasing G3P concentrations (Fig. 4). It is therefore difficult to decide by inspection whether the linear plots are really parallel or distantly convergent.

Figure 2. Effect of pH on the activity of G3P NADP⁺ reductase. The enzyme was assayed as in "Materials and Methods" in Mops 40 mM, Tricine 40 mM, EDTA 1 mM at the pH indicated (adjusted with KOH). Substrate concentrations were: 150 μM G3P, 100 μM NADP⁺ (○); and 30 μM G3P, 10 μM NADP⁺ (○).

Figure 3. Nonhyperbolic effects of substrate concentration on GNR reaction rate. A, NADP⁺ varied at 4.5 μM G3P (○) or 100 μM G3P (●). B, G3P varied at 3 μM NADP⁺ (○) or 50 μM NADP⁺ (●). Velocity expressed as nmol min⁻¹ ml⁻¹ of enzyme (5 μg protein ml⁻¹). Same set of experiments as in Figures 4 and 5. In this and Figures 4 and 5, only part of the original data and plots (68 assays) are shown for clarity.

Figure 4. Double-reciprocal plots of GNR reaction rate with varying NADP⁺ concentrations at several fixed concentrations of G3P (indicated by numerals, μM).
Symmetrically, G3P concentration versus velocity plots (Fig. 3B) were hyperbolic only at NADP\(^+\) concentrations exceeding 10 \(\mu\)M, and showed increasing inhibition with decreasing NADP\(^+\). The overall pattern is better illustrated by \(1/[G3P]:1/v\) plots (Fig. 5). This is reminiscent of substrate inhibition by G3P except that \(1/V_m\) intercepts are finite values, and the pattern will be referred to as a 'G3P-inhibited state' of GNR. Extrapolated \(V_m\) values of Figure 5 essentially fit the curve obtained with a [G3P] of 100 \(\mu\)M in Figure 3A. The intercepts (\(1/V_{app}\)) of Figure 4 fitted a limiting \(1/V_m\) plot parallel to other lines in Figure 5, allowing the limiting \(K_m\) G3P to be extrapolated. \(K_m\) G3P was 20 to 40 \(\mu\)M in several preparations, and \(K_m\) NADP was 2 to 5 \(\mu\)M.

Product inhibition patterns indicated linear competitive inhibition by NADPH when NADP\(^+\) was varied, regardless of G3P concentration (Fig. 6), and mixed-type inhibition when G3P was varied, becoming uncompetitive at saturating NADP\(^+\) concentrations (>20 \(\mu\)M, not shown). \(K_i\) NADPH, representing a dissociation constant, was 30 \(\mu\)M. Notably, nonlinear double-reciprocal plots tended to become linearized in the presence of high NADPH (Fig. 6).

The product 3PGA behaved as a mixed-type inhibitor when NADP\(^+\) was varied at fixed G3P concentrations (Fig. 7), or G3P was varied (Fig. 8). \(V_m\) versus [3PGA] replots yielded a \(K_i\) 3PGA of 10 mm (not shown). Inhibition by 3PGA was partially relieved by saturating NADP\(^+\) (Fig. 7). No activity was observed when 3PGA (0.1-50 mm) and NADPH (10-300 \(\mu\)M) were assayed together in the absence of substrates of the forward reaction, considered to be irreversible (19).

A number of compounds of possible physiological significance have been examined as modulators of GNR, using assays at high and low substrate concentrations. Table III lists...
or formed when (Table III). Exert phosphates including most and 6-phosphate; 2,6-bisphosphate; tose mm. sugar Table and mined; PEP, 19 for NADP+ IM A'. The assays contained 100 μM G3P with 100 mM F2,6BP, sedoheptulose 7-phosphate. 1,6-bisphosphate; FBP, fructose 1,6-bisphosphate; E4P, fructose 2,6-bisphosphate; S7P, sedoheptulose 7-phosphate.

Table III. Effect of Inhibitors on GNR Activity

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>A (A')</th>
<th>B (B')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi</td>
<td>94</td>
<td>80</td>
</tr>
<tr>
<td>PPI</td>
<td>ND</td>
<td>96</td>
</tr>
<tr>
<td>3PGA</td>
<td>81</td>
<td>72</td>
</tr>
<tr>
<td>PEP</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>DHAP</td>
<td>ND</td>
<td>98</td>
</tr>
<tr>
<td>E4P</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>E4P (0.03 mM)</td>
<td>ND</td>
<td>17</td>
</tr>
<tr>
<td>R5P</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td>Ru5P</td>
<td>ND</td>
<td>83</td>
</tr>
<tr>
<td>G6P</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>FBP</td>
<td>102</td>
<td>100</td>
</tr>
<tr>
<td>F2,6BP</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>S7P</td>
<td>95</td>
<td>77</td>
</tr>
</tbody>
</table>

* (A'), data from ref. 17 for Chlamydomonas enzyme and (B'), ref. 19 for pea shoot enzyme, under similar conditions; ND, not determined; PEP, phosphoenolpyruvate; DHAP, dihydroxyacetone phosphate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; G6P, glucose 6-phosphate; FBP, fructose 1,6-bisphosphate; F2,6BP, fructose 2,6-bisphosphate; S7P, sedoheptulose 7-phosphate.

several sugar phosphates, most of which exert little effect at 3 mM. Previous results (17, 19) are also shown for comparison and most are in good agreement. Heptose and hexose phosphates including the regulatory compound, fructose 2,6-bisphosphate, are ineffective or only weakly inhibitory. Pentose phosphates exert some inhibition, e.g. ribose 5-phosphate (Table III).

Erythrose 4-phosphate is a potent inhibitor of the GNR (19) and has been studied in detail. It is inactive as substrate. Double-reciprocal plots indicate simple competition by E4P against G3P (Fig. 9). Basically uncompetitive inhibition is observed when NADP+ is the varied substrate in the presence of different E4P concentrations (Fig. 10). However, at very low G3P and high E4P concentrations, reaction rates increase if the NADP+ is very high and the patterns then become biphasic (compare Fig. 7 for 3PGA). Apart from this complicating effect, secondary plots of 'slope',NADP+ versus E4P are linear with a K of 7 μM, representing the dissociation constant of enzyme/E4P complex. Dihydroxyacetone phosphate is weakly inhibiting (Table III).

The nonphosphorylated substances tested including pyruvate, glyoxylate, citrate, malate, and several other dicarboxylates, and sulfhydryl compounds including DTE have no effect on reaction rate (not shown) although the GNR contains essential thiols (16). Besides sugar phosphates, some other phosphate compounds inhibit the GNR at reasonably low concentrations. Inorganic phosphate inhibits competitively towards G3P (Fig. 11) and uncompetitively towards NADP+ (Fig. 12) with a K of 2.4 mM. Pi is increased when equimolar MgCl2 is added (not shown). Under "3G3P-inhibited" conditions the Pi may linearize the responses thereby increasing reaction rates at low NADP+ concentrations. Moreover, when G3P is low and Pi is high, velocity abruptly increases at saturating NADP+ (Fig. 12) as noted above for 3PGA and E4P. Phosphoenolpyruvate was mixed-type inhibitor to G3P with a K of 2.2 × 10^-3 M under assay conditions. Unexpectedly, pyrophosphate was without effect (Table III).

As illustrated in Table IV, free adenylates are inhibitory. ATP (K = 1.4 mM) and ADP (K = 2.0 mM) acted competitively toward G3P. The effects were dramatically decreased by Mg ions, which were slightly inhibitory when applied alone. GNR inhibition by free ATP and ADP was however nonhyperbolic under some conditions suggesting conformational effects, and was not studied further.

**DISCUSSION**

**Reaction Mechanism**

Initial velocity studies of GNR reported above have been performed in the range of substrate concentrations 1.0 to 500

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Figure 9. Competitive inhibition by E4P against G3P. Assays performed by varying G3P at a fixed NADP+ concentration (10 μM), with or without E4P. Inhibitor concentrations (μM) are indicated. Inset, secondary plot of slope, versus [E4P] yielding K[E4P] = 7 μM.

Figure 10. Uncompetitive inhibition by E4P (concentrations indicated, μM) in 1/[NADP+]:1/v plots at a fixed G3P concentration (11.2 μM). Downward bending of the plots is apparent at high NADP+, high G3P, and high E4P concentrations. Inset, secondary plot 1/v versus [E4P].
μM. The responses to the substrates NADP+ and G3P are hyperbolic over part of this range, and double-reciprocal plots are partly linear and with essentially parallel patterns. Our data therefore are inconsistent with findings of intersecting patterns and the interpretation of a rapid equilibrium random mechanism (16), whereas the limiting $K_m$ values are similar. The reason for the discrepancy is unclear, although in the work of Iglesias and Lossada (16), substrates were used at high concentrations and this may have concealed some nonlinear effects.

Dead-end and product inhibition patterns also fail to support a rapid equilibrium random mechanism (25). 3PGA acts as a mixed-type inhibitor with respect both to G3P and NADP+, and NADPH is competitive with NADP+ but mixed with G3P. Other dead-end competitors of G3P (Pi and E4P) appear to be basically uncompetitive towards NADP+. An ordered or a ping-pong mechanism would be in accord with most results. However, inhibition by 3PGA on G3P in the ping-pong case would be competitive, not mixed, except in the less likely assumption of a secondary dead-end inhibition

$$\text{(D-G3P)}_m^*$$

![Figure 11](image1.png)

**Figure 11.** Competitive inhibition by inorganic phosphate in 1/[G3P] :1/v plots at fixed NADP+ (10 μM) Pi concentrations (mm) are indicated. Inset, secondary plot of slope $\text{[Pi] vs } \text{[Pi]}$. The intercept is $K_0(\text{Pi}) = 2.4$ mm.

Site of 3PGA. Consequently, the more probable reaction mechanism within the range of linear kinetics is ordered (Scheme 2).

An ordered mechanism is also compatible with the effects of NADP+ on isolated GNR, e.g. shortening of the lag phase during assays (16) and stability (Table II). Since primary plots are almost parallel, $K_a(\text{NADP}^+)$ must be well below $10^{-6}$ M and $K_e(\text{G3P})$ may be in the low μM range. However, a ping-pong mechanism cannot be ruled out completely.

A newly described feature of GNR is its nonhyperbolic response at low NADP+ and moderate-to-high G3P concentrations, the ‘G3P-inhibited state’. The patterns (Fig. 3) are sigmoidal when NADP+ is varied and show inhibition at increasing G3P concentrations when G3P is varied. This is not due to substrate inhibition, however. True substrate inhibition would result in linear 1/[NADP+]:1/v plots; it would occur at G3P concentrations well above $K_m$ and lead to zero velocity at high G3P, but none of these features occurs. The limiting 1/[NADP+]:1/v plot at infinite G3P concentration resembles a parabola (Fig. 4).

Our results could be treated, in principle, by different theoretical approaches including allosteric mechanisms. They can also be explained on the basis of a NER mechanism; in fact, they closely resemble the patterns described by Ferdinand (12). In the NER case, the linear portions of the plots suggest an ordered reaction can be regarded as a limit situation of a general, nonhyperbolic pattern. Moreover, the products of the measured kinetic constants would be a constant, i.e. $K_a(\text{NADP}^+) \times K_m(\text{G3P}) = K_m(\text{NADP}^+) \times K_e(\text{G3P})$. Both products, indeed, may have a value in the range of $10^{-11}$ M (see above). Unfortunately, neither this constant nor confirmatory Haldane relationships can be calculated exactly, also because the reverse reaction is zero.

The proposed alternative, slower kinetic pathway postulated for a NER mechanism, is shown in Scheme 3.

Under ‘G3P-inhibited’ conditions of catalysis, NADPH

$$\text{NADP}^+ \xrightarrow{\text{G3P}} \text{E} \xrightarrow{\text{E-NADP}^+} \text{G3P} \xrightarrow{\text{E-NADP}^+} \text{E-NADP}^+ \rightarrow \text{E} \rightarrow \text{NADPH-PGA} \rightarrow \text{NADPH-PGA} \rightarrow \text{E}$$

![Scheme 2](image2.png)

**Scheme 2**

### Table IV. Inhibition of GNR activity by 5 mM adenylate compounds (titrated to pH 8 with Tris), and effect of MgO2 (10 mM)

<table>
<thead>
<tr>
<th>Adenylate compound</th>
<th>A, high substrates</th>
<th>A, high substrates + Mg</th>
<th>B, low substrates</th>
<th>B, low substrates + Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>65.2</td>
<td>92.2</td>
<td>26.5</td>
<td>62.6</td>
</tr>
<tr>
<td>ADP</td>
<td>69.4</td>
<td>89.1</td>
<td>27.2</td>
<td>65.3</td>
</tr>
<tr>
<td>AMP</td>
<td>77.4</td>
<td>82.2</td>
<td>32.0</td>
<td>74.4</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>91.0</td>
<td>100</td>
<td>87.4</td>
</tr>
</tbody>
</table>

* A, assayed with 150 μM G3P and 100 μM NADP+; B, assayed with 30 μM G3P, 10 μM NADP+.
(Fig. 6), Pi (Fig. 12), and other competitive inhibitors of G3P (not shown) can somewhat increase GNR activity by restoring hyperbolic responses. This effect, too, may be interpreted in terms of NER mechanism. Enzyme-bound NADPH may lock the protein in a state unable to initiate the alternative pathway, while competitive inhibitors may displace G3P from the enzyme site. Both effects would favor the normal pathway (NADP* binding first) and hyperbolicity. However, the NER hypothesis will remain speculative until more is known about GNR properties and reaction intermediates (16). The non-linear enhancement of activity by competitive inhibitors of G3P (Pi and E4P, Figs. 10 and 12), occurring at low G3P and high NADP* concentrations, remains to be investigated. Similar inhibition patterns have been reported for other random reactions (6).

**Physiological Role of GNR**

The GNR reaction in vivo appears to be finely regulated, primarily by the product NADPH (K_i = 30 μM). When G3P occurs at low levels, the enzyme kinetics are linear and rates are low. When G3P increases, the 'G3P-inhibited state' may set in. This latter situation may be uncommon since NADP*, NADPH, and competitive inhibitors of G3P can relieve the 'G3P-inhibited state' of GNR. Therefore, the ordered mechanism and hyperbolic responses are likely to be prevailing in vivo.

In the absence of appropriate control mechanisms, substantial amounts of triose phosphate could be transformed to 3PGA by the GNR since the substrate affinity (limiting K_m G3P = 20–40 μM), AG^*, and K_i 3PGA are so elevated. This would restrict ATP synthesis by the NAD-triose phosphate dehydrogenase/3PGA kinase pathway as well as sucrose synthesis at the level of aldolase, since K_m G3P is 0.2 to 0.3 mm for both of these enzymes (10, 21). The GNR reaction, in a sense, is energetically dissipatory and its full activity seems better compatible with the high-energy status of a photosynthesizing cell. Like glucose 6-phosphate dehydrogenase (11), GNR activity may only be allowed by de-inhibition. The GNR reaction also results in lowered pH and it may contribute in a sensitive fashion to damping out of cytosolic pH increase during photosynthesis. Activity is strongly stimulated with increasing pH between 7 and 8 (Fig. 2) (18), as in the case of other acid-producing enzymes (7).

E4P, an intermediate of the pentose phosphate pathway, is an analogue of G3P and a powerful inhibitor of GNR with a K_i of 7 μM. Ribose 5-phosphate could also contribute to the inhibition, but only at mM levels (Table III). The cytosolic pentose phosphate pathway seems to be repressed in the light (1), therefore GNR inhibition by E4P and ribose 5-phosphate may be negligible during leaf photosynthesis. Inorganic phosphate inhibits GNR at mM concentrations, which often occur in the green cell (8, 21), while being a substrate for NAD-triose phosphate dehydrogenase (K_m 0.4 mm) (10). We can attempt to outline two simplified metabolic scenarios.

Under high photosynthesis conditions, cytosolic triose phosphate concentration is high and Pi is low (26). GNR activity is at its height and controlled primarily by NADPH turnover rate. The equilibrium reaction of NAD-triose phosphate dehydrogenase/3PGA kinase is limited by Pi availability and ATP turnover. Since the ATP requirement for sucrose synthesis can be supported by mitochondrial glycine oxidation (13), catalysis by NAD-triose phosphate dehydrogenase in C_3 species is likely to be sluggish. The antagonistic roles of GNR and NAD-triose phosphate dehydrogenase during photosynthesis has been highlighted in phosphate-limited cell cultures (9). On the other hand, cytosolic Pi concentration in darkness is high and the ATP/ADP ratio is low. Oxidation through NAD-triose phosphate dehydrogenase is stimulated. GNR activity is held in check by the low [G3P]/[Pi] ratio and presence of E4P and NADPH produced by the pentose phosphate pathway. This state is often associated with an increased level of fructose 2,6-bisphosphate (21), but this modulator does not affect GNR.

The regulatory role of ATP, ADP, and AMP (Table IV) is unclear. A free ATP pool could inhibit both GNR and NAD-triose phosphate dehydrogenase (5). If respiration is hampered by slow utilization of high-energy compounds, substantial build-up of free ATP (and phospho-enolpyruvate) may potentiate GNR inhibition, although K_i is at mM levels for both compounds. Iglesias et al. (17) found little inhibition by adenylates in *Chlamydomonas GNR*.

Specific metabolites therefore appear to regulate the partitioning of oxidative carbon flow through NAD-triose phosphate dehydrogenase and GNR, in agreement with the concept of a GNR-dependent shuttle of photosynthetic NADPH to the cytoplasm (3). The importance of GNR as an NADPH-producing enzyme in the cytosol is more readily understood for specialized biosynthetic tissues (4, 18) than for normal leaves, although these are a general source of export metabolites (24). However, hydroxypropyruvate reductase is partly cytosolic and NADPH-dependent (14, 20), and the NADPH produced by GNR could act as a photorespiratory reductant. GNR is absent from bundle sheath cells of maize (28), which are deficient in triose phosphate production and photorespiration.

To the extent that leaf GNR activity is matched to photorespiratory needs, the reaction product 3PGA^2- must return to plastids via the Pi carrier to maintain the carbon balance (19). GNR is well suited to such a cycling role due to its high ΔG^* and limited inhibition by 3PGA.

**LITERATURE CITED**